The obtained uptake data has been correlated with particle composition and changes in size, allowing for comparisons among affinity baits and their effect on the interior environment of pNIPAm particles.

Citations:
[1] Longo C, Patanarut A, George T, Bishop B, Zhou W, et al. (2009) Core-Shell Hydrogel Particles Harvest, Concentrate and Preserve Labile Low Abundance Biomarkers. PLoS ONE 4(3): e4763. doi:10.1371/journal.pone.0004763

3139-Pos

UV Laser Patterning for Biocompatibility Control of Polystyrene

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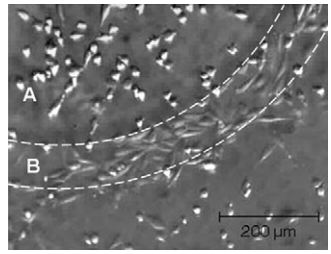
The control of cell adhesion at polymer surfaces is of great interest for applications in medicine and biotechnology research.

We present a method of polystyrene (PS) surface treatment by UV laser irradiation (laser wavelength λ=193 nm) for the improvement of adhesion of Chinese hamster ovary (CHO) cells.

We irradiated the PS foils with a total fluence of 200 mJ/cm2 in a circular spot. The irradiation led to formation of specific micro pattern at the surface (assessed by AFM and SEM). Depending on the position at the spot, the pattern structure varies. There is a specific ring area (B in figure), where seeded CHO cells show effective adhesion and pronounced spreading. The cells display here a preferential alignment along the ring. The topography of this area has a height variation of 5 to 10 nm, while the surface region irradiated with higher laser intensity (A in figure) has a higher roughness of hundreds of nm. This area is less favorable for CHO cells adhesion as indicated by the round cell morphology, similar to the flat area outside the ring. (Supported by the Austrian NANO Initiative in the projects NSI_NBPF and NSI_PolyModEUV.



3140-Pos

A Microfluidic Device for Generating Titration Curves of Biomolecular Interactions

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We present a microfluidic device for measuring affinities of biomolecular interactions. Previous work in our group has led to the development of microfluidic mixing devices, a method for biophysically characterizing molecular interactions, a platform for in situ protein expression from arrays of DNA templates, and the ability to independently address rows of the array. By combining these techniques, we can generate titration curves for a single species of a fluorescently labeled molecule against as many as 48 interaction partners in a single experiment. This approach consumes less than a picomole of the labeled molecule per titration curve, of particular use when the material is precious and of low abundance.

3141-Pos

Using Magnetic Fluids as a Versatile Method for Manipulating and Sorting Unlabeled Nonmagnetic Particles in a Flow

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Sorting and detection technologies have become an important part of industrial and medical practice. Recently, innovation in lab-on-a-chip technologies promises smaller, less expensive, and more portable devices for these applications. Labeling efficiency, specificity and throughput are challenges that must be overcome in developing such technologies. We introduce a continuous-flow magnetic flow focusing, sorting and detection scheme for unlabeled particles on the size order of cells. Unlabeled particles are focused and sorted by size in the apparatus using the magnetophoretic force in a specially crafted high-gradient magnetic field. The magnetic scheme is orthogonal to other sorting techniques, allowing other physical properties to be explored. We demonstrate using the light pressure force from a laser to actively sort a focused stream of flowing particles and use the balance between the light pressure force and the magnetic force as an additional physical axis on which particles can be sorted. We show that the positions and distribution of the particles conform to their theoretical expectations, and use the theory to explore the limitations of this technology in practice.

3142-Pos

Detection and Localization of Specific Sequences on Single Microfluidically Trapped DNA Molecules using PNA Probes

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We propose a single molecule fluorescence-based approach to rapidly locate specific sequences on DNA. Using the roughly 50,000 base pair lambda-DNA as a model molecule, we demonstrate that patterns of targeted sequences can be detected using peptide nucleic acid (PNA)-based probes. These bisPNAs, modified with biotin and Tamra on opposing ends, bind to target sequences on double-stranded lambda-DNA. While PNA probes were chosen for their specificity and versatility, they are prone to bind to non-target sites that differ from the target site by one terminal base pair. PNA binding to these single-end mismatch (SEMM) sites can be minimized by a moderate amount of additional heating following the binding reaction and this step must be optimized to achieve the requisite specificity.

Here we demonstrate the single-molecule analysis of the binding of two PNAs, with three and two target sites on lambda-DNA, respectively. Neutravidin-coated 40 nm fluorescent polymer spheres are attached to the DNA-bound biotinylated PNAs and the DNA is fluorescently stained. The locations of the bound beads along single DNA molecules are determined by stretching the DNA on slides and by trapping and stretching single DNA molecules in a microfluidic cross-slot, utilizing a stagnation-point extensional flow. In comparing to previously published results, we find that the end modifications have substantial effects on binding conditions. Furthermore, the effects of additional heating on individual target sites and mismatched sites were quantified.

3143-Pos

Suppressing Non-Specific Interactions Between Solid Surfaces used for Single-Molecule Force Measurements

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Magnetic tweezers (MT) are broadly used for investigating interactions involving nanometer-sized molecular complexes, using micrometer sized super paramagnetic beads. In contrast to other force techniques such as optical tweezers and atomic force microscopy, magnetic tweezers offer key advantages: (1) MT allows for recording of hundreds of single-molecule events in parallel with a single measurement; (2) magnetic forces are orthogonal to most biological interactions, eliminating perturbation of the sample properties during the MT measurements; (3) MT requires relatively low energy - thereby sample overheating is not an underlying problem for MT measurements; (4) the capability to conduct MT measurements at constant force eliminates the need for considering loading rates (*dF/dt*). Due to prevalent forces resultant from non-specific interactions between probe interfaces at nanometer separation, the utilization of single-molecule force techniques in proteomics remains largely unexplored. Employing surface-engineering methodologies, developed in our laboratory (*Ann. Biomed. Eng.***2009**, *37*, 1190-1205), we aim to establish the utility of MT for proteomics research by suppressing the non-specific interactions between superparamagnetic microbeads and flat substrates. Surfaces coated with synthetic polymers, providing entropic repulsion, allow for desorption of the beads from the flat surfaces with quantitative yields. Covalent attachment of to such interfaces does not compromise the functionality of enzymes and other proteins.

3144-Pos

Excitation of Microtubules Using a Double Slit Ultrasound Device

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Microtubules (MTs) are a major part of the cytoskeleton of all eukaryotic cells and directly contribute to the process of cell division by forming mitotic spindles and providing force for the segregation of chromosomes. In this work first we have analytically solved the problem of the vibrational dynamics of a MT that is attached at its two ends (which is relevant for MTs during the mitosis) inside a viscous solution, driven by an ultrasound plane wave. We have shown that with using ultrasound plane waves, the resonance only happens at high values of the harmonic mode number. However, due to the small amplitudes of those modes we cannot have both frequency control and energy transfer to the MT at the same time. Having a large enough amplitude for the resonant vibration effect is crucial in order to maximize the bending moment of a MT. In order to overcome this difficulty, we propose to excite the MT using an ultrasound generation device using a double slit design that allows for both the frequency control and optimized energy transfer to the MT.